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SUMMARY OF EXISTING DATA, PROPOSED TEST PLAN AND RATIONALE FOR COBALT NAPHTHENATE (CASRN 61789-51-3)

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INTRODUCTION

The following document includes a test plan and a summary of existing data for cobalt naphthenate [CASRN 61789-51-3]. The information provided in this document and the attached dossier of robust summaries meets the requirements under the U.S. High Production Volume (HPV) Chemical Challenge. Cobalt naphthenate is one of 19 sponsored chemicals organized under the Metal Carboxylates Coalition (The Coalition), an HPV testing consortium managed by the Synthetic Organic Chemical Manufacturers Association's (SOCMA) VISIONS Department. The Coalition member companies sponsoring cobalt naphthenate are OM Group, Inc., The Shepherd Chemical Company and Troy Corporation.

USE PATTERNS AND REGULATORY BACKGROUND

Cobalt naphthenate is the cobalt salt of naphthenic acids. Naphthenic acids are a complex group of carboxylic acids with the general formula $C_nH_{2n+z}O_2$, where n indicates the carbon number and Z specifies the hydrogen deficiency resulting from ring formation (Clemente and Fedorak, 2005). Cobalt naphthenate is thus a member of the metal carboxylates group.

All of the metal carboxylate salts are designed to add metals to chemical reactions. They therefore are expected to dissociate into free metal and free acid.

In general the cobalt carboxylates are used as oxidative polymerization catalysts in many product areas. These areas include but are not limited to: ink and paint driers; unsaturated polyester resins, and hydrodesulfurization in their manufacturing; and the making of the insecticide DEET (diethyltoluamide). Some of these carboxylate compounds are used in oxygen scavenger plastics as well (for example, plastic bottles). The tire industry also uses cobalt carboxylates as adhesion promoters in tire manufacturing. These compounds facilitate adhesion between the rubber in the steel cords. The metal (not salt) loadings range from 0.01-0.5% depending upon the application listed above.

Cobalt naphthenate is used primarily in unsaturated polyester resins, paint drier applications and rubber adhesion promotion in the tire industry.

One characteristic of cobalt naphthenate and other metal carboxylates is that they readily dissociate from an ion pair into free metal and free acid. They are found as partially dissociated products in the ambient environment (i.e., neutral pH). Dissociation is a reversible process and the proportion of dissociated salt is dependent on the pH and pKa (the dissociation constant), which is the pH at which 50% dissociation occurs. In the low pH environment of the digestive tract

(e.g., pH 1.2) complete dissociation will occur for these metal carboxylates. The transport and bioavailability of the metals and acids are determined by their solubility in environmental media and biological fluids which is determined by environmental parameters such as pH.

Dissociation is a reversible reaction, splitting the parent compound into two or more chemical species which may be ionic, but are not necessarily so. The process can be generally represented as:

$$[RCOO^-]_x{:}[M^+] \overset{pK1}{\leftrightarrow} [RCOO^-]_x + [M^+] \overset{pK2}{\leftrightarrow} [RCOOH]_x + [M^+]$$

undissociated salt (High pH) — **dissociated acid and metal** — **neutral species + ionized metal** (Low pH)

The pKa and pH are equal when the metal carboxylate salt is 50% dissociated. The parent compounds, the metal carboxylate salts, are associated ionized molecules.

The Metal Carboxylates Coalition conducted a study following OECD Guideline 112 to determine the dissociation constant of cobalt naphthenate. The mean pKa values were 6.74 and 8.00 at 20ºC. This result indicates that a moderate amount of dissociation will occur at approximately neutral pH (i.e., representative of aquatic and marine ecosystems), while complete dissociation will occur at the physiologically relevant pH of the mammalian stomach (pH 1.2). These findings are particularly important in relating available data for naphthenic acids and cobalt to support the existing data for cobalt naphthenate in the fulfillment of critical endpoints.

Because the free acid (naphthenic acid) and corresponding free metal (cobalt) have different characteristics (e.g., solubility, adsorption, and toxicity) than the undissociated salt (ion pair), the proportion of dissociation influences the behavior of the substance in the environment and *in vivo*. The bioavailable fraction of the constituents of metal carboxylate salts can be estimated from the dissociation constants.

There are two principal hazard assessments being evaluated based on the current data for cobalt naphthenate. The first is the hazard to aquatic organisms due to environmental exposure. The second is hazard to mammalian systems as a result of oral exposure. Based upon the pKa of 6.74 and 8.00, it is expected that in the ambient aquatic environment, moderate portions of the cobalt naphthenate will be dissociated; therefore, part of the compound will be present as naphthenic acid and cobalt cations. In the environment (i.e., aquatic systems), toxicity is typically related to the free metal ion concentration (U.S. EPA, 2002). The metal ion pair (salt) is less likely to be absorbed and to contribute to toxicity.

At the low pH of the mammalian stomach (pH 1.2) all of the metal carboxylates, including cobalt naphthenate, are expected to be completely, or nearly completely, dissociated. This indicates that when administered orally, the absorption and resulting toxicity would be due to the independent action of the naphthenic acid and the free (ionized) cobalt. This is supported by *in vivo* and *in vitro* data with cobalt acetate and other cobalt containing carboxylates (Firriolo 1992.; Speijers et al 1985; Stopford et al. 2003) (See discussion below).

The dissociation constant shows that at the pH of the stomach, the important moieties from a toxicological standpoint are the unionized free naphthenic acids and ionized cobalt. Because of this dissociation in the stomach, mammalian toxicity data for naphthenic acid can to serve as a surrogate data for the carboxylic acid component of cobalt naphthenate. Similarly, under these conditions, data for cobalt can be represented by fate and toxicity data for free ion or simple metal salts (e.g., cobalt chloride). Therefore, the role in any observed toxicity for acids and metals can be evaluated independently.

Bioequivalency

The work described below by Stopford et al. (2003) shows that cobalt chloride is similar to, or more bioavailable than, the corresponding cobalt carboxylate salts, which makes the chloride a conservative surrogate in estimating bioavailability and toxicity of dissociated metal. Cobalt chloride has thus been emphasized during preparation of the attached robust summaries and provides the preferred surrogate data for cobalt carboxylate salts, including cobalt naphthenate.

The recent studies by Stopford et al. to evaluate the "bioequivalency" (an estimate of bioavailability) of cobalt compounds included three cobalt carboxylates, including cobalt naphthenate, and cobalt chloride (when added as fine powders) in synthetic fluids designed as surrogate gastric juices. These investigators showed that these cobalt salts were completely dissociated and dissolved at a gastric pH (1.2) (Table 1). When added to surrogate intestinal fluids at neutral pH (7.4), $Co(II)Cl_2$ was also highly soluble. The solubility of the cobalt (% available cobalt expressed as Co(II) ion) in cobalt carboxylates ranged from 30.8 to 50.8 percent available cobalt at 72 hours (Table 1). These results for cobalt chloride and cobalt naphthenate are highly consistent with data reported by Firriolo (1992) for these same salts in similar surrogate biological fluid matrix (Table 1). Maximum solubility of cobalt naphthenate was observed at 48 hrs, which was the longest sample time used in the study.

These bioequivalency data are valuable for two reasons. They confirm the prediction from the dissociation studies that these compounds are expected to be completely dissociated in the gastrointestinal tract (low pH) and a substantial proportion of these compounds would be expected to be dissociated and bioavailable in water at neutral pH (7.4).

Table 1: Results of extraction of cobalt from surrogate biological fluids

Matrix (pH)	Maximum Solubility (% of available metal)			
	$CoCl_2$	Co 2-ethyl-hexanoate	Co naphthenate	Co neodecanoate
Gastric pH (1.5)[a]	100	100	100	100
Gastric pH (2.0)[b]	100		100	--
Intestinal pH (7.4)[a]	100	50.8*	45.4*	30.8*
Intestinal pH (7.3)[b]	85	--	20**	--

[a] From Stopford et al. (2003); [b] Firriolo (1992)

* Maximum concentration observed at 72 hours.

** Maximum concentration observed at 48 hours.

Stopford et al. (2003) and Firriolo (1992) added all of the salts to the neutral (intestinal) surrogate solutions as finely ground powder. It is not surprising that the percent of available cobalt from cobalt carboxylates appears to increase with time (48 or 72 hours). Firriolo (1992) also evaluated the solubility of ground and ethanol-solubilized cobalt naphthenate in a neutral buffer solution[1]. For ground cobalt naphthenate, 20% of available Co(II) was dissociated. In contrast, 90% of available cobalt was observed as dissociated Co(II) when originally introduced in ethanol. The ethanol-solubilized Co(II) remained in solution. This finding has implications for dissociated Co(II) introduced to the intestine solubilized in gastric juices.

Cobalt is absorbed primarily as the free Co(II) ion via biochemical mechanisms at the intestinal mucosal wall (Firriolo 1992). Having been reported as completely soluble in gastric fluids (Stopford et al. 2003; Firriolo 1992), Co(II) should remain soluble (100% dissociated Co(II)) after entering the intestine from the stomach. Once solubilized, this cobalt would be expected to undergo the same fate irrespective of the salt originally ingested. Stopford et al. (2003) emphasized the importance of confirming the interpretation of *in vitro* solubilities in surrogate fluids with *in vivo* data. In fact, Firriolo used these (Table 1) *in vitro* solubility tests as preliminary studies for subsequent comparative absorption, distribution and elimination studies. Discussion of *in vivo* data is presented in the following section.

Finally, the work by Stopford et al. (2003) shows that the metal chloride is similar to, or more bioavailable than, the corresponding metal carboxylate salts (Table 1), which makes the chloride a conservative surrogate when attempting to estimate the bioavailability and toxicity of dissociated metal salts. For this reason, data for the chlorides of cobalt have been emphasized during preparation of the attached robust summaries and is the preferred surrogate for the cobalt dissociation product of cobalt naphthenate.

[1] PBS = phosphate buffered solution without $CaCl_2$ or $MgCl_2$

Comparative Toxicity and Pharmacokinetics

Toxicity data for soluble cobalt salts indicate that the contribution of the respective anion to the toxicity of the compound is negligible compared with that of the cobalt cation. Speijers et al. (1982) investigated the acute oral toxicity in rats of a series of cobalt compounds including cobalt acetate. Lethal doses varied significantly when calculated in terms of the compound weight; however, when based on the dose of the Co(II) ion, all of the LD50 values were within a factor of about two for all of the compounds (Table 2). With the exception of the fluoride and bromide salts, all other salts tested had LD50 values within the range from 140 to 190 mg Co/kg bw. The LD50 for cobalt acetate was in the middle of this range at 168 mg Co/kg bw. This work indicates that toxicity is related to the cobalt ion and independent of counter ions. Similar results would be expected for cobalt naphthenate.

Table 2. A comparison of acute oral toxicity values of cobalt compounds calculated based on the weight of each compound or on the cobalt content of each respective compound.

Compound	LD50* (mg compound /kg bw)**	LD50* (mg Co/kg bw)
Cobalt(II) fluoride	150	91
Cobalt(II) oxide	202	159
Cobalt(II) phosphate	387	187
Cobalt(II) bromide	406	109
Cobalt(II) chloride	418	190
Cobalt(II) sulphate	424	161
Cobalt(II) nitrate	434	140
Cobalt(II) acetate	503	168

* Data from Speijers et al. (1982)
** Several test compounds were hydrates and contained water. Results are expressed based on the weight of the anhydrous compound.

This toxicity data is supported by evaluation of the absorption, distribution, and elimination of cobalt following exposure to different metal salts. Work by Firriolo et al. (1999) showed that regardless of whether the compound was introduced as Co(II) chloride or Co naphthenate, the absorption, disposition, and elimination of cobalt was the same. This data indicates that the carboxylic acid portion of the salt does not play a role in cobalt ion absorption *in vivo* once the compound (ion pair) has dissociated. These authors state that absorption of cobalt in the GI tract is dependent upon release of free metal ion and their results indicate that the acid, in this case naphthenate, does not limit the degree of absorption.

Firriolo et al. (1999) confirmed previous findings that cobalt absorption occurs in the jejunum of the small intestine. Working with intestinal rings, these authors

showed that absorption of cobalt occurred via biochemical processes that occurred at the intestinal mucosal wall. These processes appear to be saturable and both concentration and temperature dependent (Firriolo, 1992). These characteristics are indicative of active transport (Ashmead et al., 1985 and Firriolo et al., 1999). In addition, there appears to be a diffusional component to the absorption of cobalt ions, which is also concentration dependent (Firriolo et al., 1999). Despite the presence of these mechanisms for cobalt absorption, uptake from the gut is incomplete. Only limited absorption of ingested cobalt occurs (e.g., 20% – 30%) in the gut (Firriolo, 1992; ASTDR, 2001).

The *in vivo* toxicity (Speijers et al., 1982) and absorption/distribution data (Firriolo et al. 1999) are supported by the *in vitro* data for a broader range of cobalt carboxylates (Stopford et al., 2003; Firriolo, 1992; Firriolo et al., 1999). This body of work shows that the hazard of these metal carboxylates is largely dependent on the metal, and not the carboxylic acid. This facilitates the use of toxicity data for soluble metal salts (e.g., $Co(II)Cl_2$) that dissociate rapidly and completely under physiological conditions, to estimate the potential hazard of cobalt naphthenate.

Supporting Data for Dissociation Products

Consistent with discussions between the Metal Carboxylates Coalition and the EPA, data for the dissociation products (metals and acids) are recognized as being essential to understanding the environmental fate and toxicological characteristics of the respective metal carboxylate salts. Data for naphthenic acids and cobalt chloride are therefore useful in characterizing the hazard of cobalt naphthenate.

In summary, the key points relative to cobalt naphthenate are:

- Dissociation to naphthenic acids and cobalt (described as cobalt chloride);
- Dissociation constant (pK values) in the circum neutral range;
- Complete or nearly complete dissociation at gastric pH (1.5 to 2.0);
- A moderate amount of dissociation in the environmental pH range (neutral);
- Existing data for the parent molecule or its dissociation products will be sufficient to address specific endpoints.

Data for cobalt naphthenate and its dissociation products are provided as follows:

1. Data for cobalt naphthenate are provided in robust summary format in Appendix A.

2. In addition, when available, data for the dissociation products (naphthenic acids and cobalt chloride) are provided.

    a. Appendix B contains a synthesis of robust summaries for naphthenic acids. Robust summaries prepared by the American Petroleum Institute for "Reclaimed Substances: Naphthenic Acids" are attached as Appendix C.
    b. Appendix D contains robust summaries for cobalt chloride.

Naphthenic Acids

Naphthenic acids are a family of carboxylic acids, composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids with smaller amounts of acyclic aliphatic (paraffinic or fatty) acids. Aromatic olefinic, hydroxyl, and dibasic acids are also present as minor components of naphthenic acids. The cycloaliphatic acids include single rings and fused multiple rings. The carboxyl group is usually bonded or attached to a side chain rather than directly to the cycloaliphatic ring (Headley and McMartin, 2004).

The robust summaries for naphthenic acid were derived from recent literature as well as information in robust summaries prepared by the American Petroleum Institute. In addition, these data are summarized and referenced in the appropriate remarks sections for each data element in the robust summaries of cobalt naphthenate. Data for naphthenic acids are discussed in the next section and summarized in Table 3.

Cobalt

Cobalt is a naturally-occurring element that has properties similar to those of iron and nickel. It is an essential element, required for good health in animals and humans (ASTDR, 2001). A biochemically important compound containing cobalt is vitamin B_{12} or cyanocobalamin. For most people, food is the largest source of cobalt intake. The average person consumes about 11 micrograms of cobalt per day in their diet (ASTDR, 2001). Part of this cobalt comes from vitamin B_{12}, which is found in meat and dairy products. Cobalt is also found in surface and groundwater. In the U.S., concentrations in water are usually between 1 and 10 μg/L (ppb), although they may be much higher in areas that are rich in cobalt-containing minerals or in areas near mining or smelting operations. In most drinking water, cobalt levels are less than 1 – 2 ppb (ASTDR, 2001).

Soluble forms of cobalt, such as cobalt(II) chloride (or cobaltous chloride), are most likely to be absorbed and cause systemic effects in humans. For this reason, this compound has often been used in absorption and toxicology studies to determine the potential hazard of cobalt exposures. When coming into contact with water and biological fluids, cobaltous chloride dissolves and releases cobalt

as a +2 ion. In general, it is the cobalt ion that is responsible for causing toxicity[2]. Because of this, in this document, the toxicity of cobalt(II) chloride (expressed in terms of the cobalt ion), is used as a surrogate for the toxicity of cobalt that is released through the dissociation of the cobalt naphthenate.

Approximately 13-34% of cobalt(II) chloride is absorbed in the gut of rats. Absorption may be increased in iron deficient individuals. The highest concentration of absorbed cobalt is in the liver and then the kidney. There is no accumulation of cobalt with age. Following oral exposure, cobalt is eliminated primarily in feces (the unabsorbed fraction) and secondarily in urine (the absorbed fraction). For cobalt(II) chloride, 70 - 80% of the administered dose is eliminated in the feces. Elimination is biphasic or triphasic. The terminal phase involves a very small residual level of cobalt and has a half-life in years (ATSDR, 2001).

The robust summaries for cobalt chloride were derived largely from well recognized and peer reviewed compendia (e.g., ATSDR Toxicological Profiles, WHO Environmental Health Criteria). These data are presented in Appendix D. In addition, these data are summarized and referenced in the appropriate remarks sections for each data element in the robust summaries of cobalt naphthenate. Data for the soluble/dissociable forms of the metal (free metal or the chloride salt) are discussed in the next section and summarized in Table 3.

EXISTING DATA FOR COBALT NAPHTHENATE AND DISSOCIATION PRODUCTS - SUMMARY

Physicochemical Properties

Available physicochemical property data for cobalt naphthenate, and for naphthenic acids and cobalt chloride, are shown in Table 3 and briefly summarized below. The sources of information for these data are given in the robust summaries (Appendixes A – D).

Product property information is available on the melting point and boiling point of cobalt naphthenate, and a GLP study was conducted to determine the water solubility. Data for all relevant physico-chemical endpoints are available for naphthenic acid and cobalt chloride (Table 3).

[2] Insoluble compounds that do not release significant amounts of the cobalt ion are much less toxic when administered orally (ASTDR, 2001). The oral toxicity of soluble cobalt compounds is similar when expressed in terms of the cobalt ion.

Melting Point

The reported melting point for cobalt naphthenate with 10.5% cobalt is 77ºC. For commercially-available naphthenic acids, the reported melting point is between -35ºC and +2ºC. The melting point reported for cobalt chloride is 735ºC.

Boiling Point

The reported boiling point for cobalt naphthenate containing 6% cobalt is 315 - 380ºC, while the boiling point for commercially-available naphthenic acids is within the range 140 - 200ºC. For cobalt chloride, the reported boiling point is 1,049ºC.

Density

The density for cobalt naphthenate is 0.9 – 0.95 g/cm^3 at 77ºC for a liquid with 6% cobalt, and 1.16 g/cm3 for a solid with 10.5% cobalt. The reported density for naphthenic acids is 0.91 – 0.96 g/cm^3 at 15ºC. The density for cobalt chloride is 3.367 g/cm^3 at 25ºC.

Vapor Pressure

Vapor pressure was not considered applicable for the salts, cobalt naphthenate or cobalt chloride. The vapor pressure for naphthenic acids was estimated using EPIWIN v.310 to be very low.

Partition Coefficient

The octanol/water partition coefficient is not applicable for salts such as cobalt naphthenate or cobalt chloride. The log octanol/water partition coefficient for naphthenic acids was estimated using EPIWIN v.310 to range from 5.1 to 9.2 depending upon the range of molecular weights present.

Water Solubility

A GLP study was conducted, following OECD Guideline 105, using the column elution method, to determine the water solubility of cobalt naphthenate. The water solubility was estimated to be 34.28 mg/L at 20ºC. The estimated water solubility for a range of molecular weight naphthenic acids ranged from 0.0003 to 2.1 mg/L. The water solubility of cobalt chloride is reported to be 450 g/L at 7°C.

Environmental Fate and Transport

Available environmental fate and transport data for cobalt naphthenate, naphthenic acids, and cobalt chloride are shown in Table 3 and briefly summarized below. The sources of information for these data are given in the robust summaries (Appendixes A – D).

Data exist for dissociation in water for cobalt naphthenate, but not for any other fate characteristics. Relevant information for the dissociation products is discussed below.

Photolysis

Experimental data are available to indicate that naphthenic acids (both mixtures and individual compounds) are not significantly degraded by exposure to natural sunlight, artificial solar radiation, or artificial UV-range radiation. The photodegradability of cobalt chloride is not relevant, as the element cobalt does not degrade further.

Dissociation in water

One key characteristic of any metal carboxylate is that it readily dissociates from an ion pair into free metal and free acid as the pH is decreased. A dissociation study was conducted according to OECD 112, under GLPs, to determine the equilibrium constant of cobalt naphthenate. The results demonstrated two pKa values, 6.74 and 8.00, at 20ºC (Lezotte and Nixon, 2002). Naphthenic acids are weak acids, with most pKa values around 5.

Biodegradation

No data are available on biodegradation of cobalt naphthenate. Experimental data are available to indicate that commercial mixtures of the sodium salts of naphthenic acids are degradable when inoculated with microbial populations indigenous to oil sands tailings. These non-guideline experiments showed degradation of 50% within 24 days (Herman et al., 1994) and 60% in 10 days (Clemente, et al., 2004). Biodegradation is not relevant for the element cobalt.

Monitoring data

No monitoring data were reported.

Transport data

Estimation of environmental transport for cobalt naphthenate is not available since fate models generally used do not accurately predict salts such as metal carboxylates. However, the distribution of a range of naphthenic acids was predicted using the Level I Fugacity model in EPIWIN v.3.10. The principal distribution following environmental release would be to soil and/or sediment, with 98% partitioning to sediment .

Ecotoxicity

Available ecotoxicity data for cobalt naphthenate, naphthenic acids, and cobalt chloride are shown in Table 3 and briefly summarized below. The sources of information for these data are given in the robust summaries (Appendixes A – D).

Fish Toxicity

There are no data on the toxicity of cobalt naphthenate to fish; however, data exist for the dissociation products.

Naphthenic acids

The 96-h TLm for zebra fish (*Brachydanio rerio*) in a static toxicity test was determined to be 16.3 mg/L. The 48-h TLm for zebra fish embryos was reported to be 3.5 mg/L. For three-spine stickleback (*Gasterosteus aculeatus*), the 96-LC50 for a commercially-available naphthenic acid mixture was estimated to be in the range of 5 mg/L. Other available data indicate a 48-h TLm of 5.6 mg/L for bluegill (*Lepomis macrochirus*).

Cobalt chloride

For cobalt chloride, the 96-h LC50 was 1.41 mg Co/L for the highly sensitive rainbow trout, *Oncorhynchus mykiss*. Other fish species were less sensitive with 96-h LC50 values ranging from 22.0 to 330 mg Co/L.

Invertebrate toxicity

There are no data on the toxicity of cobalt naphthenate to invertebrate species; however, data exist for the dissociation products.

Naphthenic acids

Toxicity data are available for non-standard test species. A 96-h LC50 of 4.8 mg/L for calcium naphthenate was reported for the marine copepod, *Nitocra spinipes*. The zooplankton species *Nephargoides maeoticus* tolerated napthenic acid concentrations only up to 0.15 mg/L.

Cobalt chloride

For cobalt chloride, reported 48-h EC50 values for *Daphnia magna* include 1.52 mg Co/L and 5.5 mg Co/L. For *Ceriodaphnia dubia*, 48-h LC50 values ranged from 2.35 to 4.60 mg Co/L.

Algal toxicity

There are no data on the toxicity of cobalt naphthenate to algae. Data exist for the dissociation products.

Naphthenic acids

The toxicity of naphthenic acids to the freshwater diatom, *Navicula seminulum*, has been measured based upon population growth. The 96-h LC50 ranged from 26.0 – 80.5 mg/L.

Cobalt chloride

For cobalt chloride, the 96-h EC50 for *Chlorella vulgaris* was 0.52 mg Co/L. For the duckweed *Lemna minor*, the 7-d IC50 was 16.9 mg Co/L, while for the blue-green alga *Spirulina platensis*, the 96-h EC50 was 23.8 mg Co/L.

There are no ecotoxicity data on cobalt naphthenate. The available data for naphthenic acids, which are based upon older non-guideline studies and non-standard species, generally indicate moderate toxicity. Cobalt chloride has moderate to low toxicity to fish and invertebrates, but appears to be highly toxic to at least some species of aquatic plants

Human Health Effects

Data are available for a number of the lower tier mammalian toxicity and genotoxicity endpoints for cobalt naphthenate. In addition, the majority of the human health effects endpoints are satisfied for the dissociation products, naphthenic acids and cobalt chloride. These data are shown in Table 3 and briefly summarized below. The sources of information for these data are given in the robust summaries (Appendixes A – D).

Acute Mammalian Toxicity

Cobalt naphthenate

Two different acute oral toxicity studies with rats produced nearly identical LD50 values (2,800 mg/kg and 2,838 mg/kg). The acute dermal toxicity of cobalt naphthenate is also low (LD50 for rabbits between 1260 and 2,000 mg/kg). No inhalation studies were available. Cobalt naphthenate was slightly irritating to the skin of rabbits and mildly or slightly irritating to the eyes of rabbits.

Naphthenic acids

Acute toxicity data are available for naphthenic acids for four of five acute endpoints (i.e., oral toxicity, dermal toxicity, skin irritation and eye irritation) as presented in Table 3. Naphthenic acids have a low order of acute toxicity. The oral LC50 is 5.88 g/kg for the rat and 3.55 g/kg for the mouse, while the dermal LD50 is greater than 3.14 g/kg for the rabbit. Naphthenic acids were moderately to severely irritating to the skin when tested on the rabbit and caused moderate eye irritation in the rabbit.

Cobalt chloride

There are extensive toxicity data available for cobalt (II) chloride and several other soluble and insoluble salts of cobalt. The single-dose rat LD50s for cobalt (II) chloride range from 140 to 190 mg Co/kg bw.. For the mouse, the LD50 value expressed as the cobalt ion is 89.3 mg Co/kg bw. Inhalation toxicity data are not available for cobalt chloride. Increased proliferation of lymphatic cells was seen in rats, mice, and guinea pigs dermally exposed to cobalt chloride in DMSO once per day for 3 consecutive days, with LOAEL values ranging from 9.6 to 14.7 mg Co/kg-day. Dermatitis, probably caused by an allergic reaction, is a common result of dermal exposure to cobalt in humans.

Repeated Dose Toxicity

Naphthenic acids

An oral 90-d subchronic toxicity test with a mixture of naphthenic acids (sodium salts) isolated from Athabasca oil sands produced significant physical, clinical, and pathological changes in rats at a dose level of 60 mg/kg/day (5 doses per week). No significant adverse effects were seen at a dose level of 6 mg/kg/day. Several parameters suggested that the liver was the primary target organ in this study. Liver weight was increased 35% above control values in the high dose group. Body weight gain was also reduced 8-9% in this exposure group compared to controls.

Cobalt chloride

Oral dosing of rats with cobalt chloride five days per week for 150 to 210 days indicated a LOAEL of 4 mg Co/kg based upon increased organ weights. This is consistent with other studies of cobalt chloride at levels ranging from 0.5 to 30.2 mg Co/kg-day. Repeated oral dosing of rats with cobalt chloride hexahydrate for 8 weeks indicated the NOAEL was 0.6 mg Co/kg and the LOAEL was 2.5 mg Co/kg, based upon changes in hemoglobin content and numbers of erythrocytes. Another study reported oral doses of 0.5 and 2.5 mg Co/kg for 7 months stimulated hematopoiesis and decreased immunological reactivity in rats, while doses of 0.05 mg Co/kg had no effects.

Genetic Toxicity – in vitro

Cobalt naphthenate

Cobalt naphthenate was generally negative in the Ames assay (one of the eight combinations of S-9 fraction and strain of *Salmonella typhimurium* tested was positive). Cobalt naphthenate was also negative in the mouse lymphoma assay.

Naphthenic acids

Calcium and sodium naphthenate were negative in the Ames assay. Sodium naphthenate was negative for chromosomal aberrations but positive for sister chromatid exchanges. Calcium naphthenate was positive in the mouse lymphoma assay.

Cobalt chloride

Cobalt compounds with a valence state of II, the form of cobalt released by dissociation of cobalt chloride, are generally non-mutagenic in bacterial assays, including plate incorporation and fluctuation assays with *Salmonella typhimurium* TA strains and *Escherichia coli* WP2. However, a weak positive mutagenic response has been found in the rec assay with *Bacillus subtilis* and in Chinese hamster V9 cells. DNA damage in isolated human lymphocytes was observed at 6.0 mg Co/L in the alkaline comet assay, and

an increase in sister chromatid exchanges has been observed in human lymphocytes and macrophages.

Genetic Toxicity – in vivo

Cobalt naphthenate
There are no *in vivo* genetic toxicity data for cobalt naphthenate.

Naphthenic acids
There are no *in vivo* genetic toxicity data for naphthenic acids.

Cobalt chloride
Oral administration of cobalt chloride hexahydrate to mice (20 – 80 mg/kg bw) produced a concentration-dependent increase in chromosomal aberrations. A dose-dependent increase in the incidence of micronucleated polychromatic erthythrocytes was observed in mice subsequent to i.p. injection of $CoCl_2.6H_20$, at doses of 25 – 90 mg Co/kg bw. Increased micronucleus formation was observed following i.p. injection of 12.4 and 22.3 mg Co/kg (as cobalt chloride), but not after injection of 6.19 mg Co/kg (NOEL).

In summary, cobalt naphthenate is generally not genotoxic, while mixed results have been demonstrated with sodium and calcium salts of naphthenic acid and with cobalt chloride.

Developmental Studies

Cobalt naphthenate
No developmental studies with cobalt naphthenate are available.

Naphthenic acids
Fetal malformations were not observed in the offspring of female rats exposed orally to naphthenic acids isolated from oil sands tailings, at a dose up to 60 mg/kg bw.

Cobalt chloride
In a developmental toxicity study with cobalt chloride exposure (5.4 to 21.8 mg Co/kg/day) in rats from gestation day 14 to lactation day 21, the LOAEL was based on stunted pup growth. However, maternal toxicity was observed in conjunction with effects on the offspring. This growth effect was considered to be a secondary or indirect effect rather than a direct effect of cobalt on the fetus. No teratogenic effects were observed. Another study in rats provided a NOAEL of 24.8 mg Co/kg/day for cobalt chloride exposure from gestation days 6-15. No effects were observed on fetal growth or survival in mice exposed to 81.7 mg Co/kg/day as cobalt chloride during gestation days 8-12.

Reproduction Studies

Cobalt naphthenate
No reproductive studies on cobalt naphthenate are available.

Naphthenic acids
An oral dose of 60 mg/kg/day of naphthenic acids isolated from oil sands tailings produced a dramatic reduction in female fertility in rats. Total cholesterol was reduced, while mating and ovulation were unaffected. A one-generation reproductive study in rabbits, using a dermal dose (2 mL) of calcium naphthenate, did not cause adverse effects on reproductive performance attributable to exposure of the males. Unexposed females mated with treated males did not have any reproductive effects, and there were no pathological findings in the male reproductive tract.

Cobalt chloride
Cobalt exposure (as cobalt chloride hexahydrate in drinking water for 12 -13 weeks) affected male reproductive parameters for mice in a time- and dose-dependent manner. All dose levels (23.0 – 72.1 mg Co/kg-day) caused decreases in testicular weight and epididymal sperm concentration. Testicular degeneration and atrophy have been reported in rats exposed to 13.2 to 30.2 mg Co/kg/day as cobalt chloride for 2-3 months in the diet or drinking water.

Other Information

Naphthenic acids
In a study in which calcium naphthenate was dermally administered to female mice (two times per day for two years), twelve epidermal and one dermal tumor at the treated sites were observed in eight of the exposed mice. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present.

Cobalt chloride
The U.S. National Toxicology Program does not recognize cobalt as a human carcinogen, but IARC has classified cobalt and cobalt compounds as possibly carcinogenic to humans (Class 2B) based on sufficient evidence that cobalt metal powder and cobaltous oxide are carcinogenic in animals. "No studies were located regarding carcinogenic effects in animals after oral exposure to stable [non-radioactive] cobalt." (ATSDR Sept 2001 Draft).

Table 3. Summary of existing data for cobalt naphthenate and its dissociation products[1]

SIDS ENDPOINT	REPORTED VALUES		
	Cobalt naphthenate	**Naphthenic acids**	**Cobalt chloride**
Physicochemical Properties			
Melting Point	77ºC for product with 10.5% Co	-35ºC to +2ºC	735ºC
Boiling Point	315ºC - 380ºC for product with 6% Co	140ºC to 200ºC	1,049ºC
Density	0.9 - 0.95 for liquid with 6% Co; 1.16 for solid with 10.5% Co	0.91 -0.96	3.367 at 25ºC
Vapor pressure	NR	Very low	NR
Log Partition Coefficient	NR	<5.1 to 9.2	NR
Water Solubility	34.28 mg/L at 20ºC	0.003 to >2.1 mg/L	450 g/L at 7ºC
Environmental Fate			
Photodegradation	--	Not significant	NR
Dissociation in water	pKa = 6.74 and 8.00 at 20ºC	Approx. 5	--
Monitoring Data	--	--	--
Transport (Fugacity)	NR	98% partitioning to soil predicted	NR
Biodegradation	--	Mixtures of sodium salts of naphthenic acids have exhibited degradation (50% in 24 days; 60% in 10 days)	NR
Ecotoxicity			
Fish toxicity (96-h LC50)	--	3.5 – 16.3 mg/L for various species	1.41 - 333 mg/L; rainbow trout most sensitive
Invertebrate toxicity (48-h EC50)	--	4.8 mg/L for calcium naphthenate (*Nitocra spinipes*)	1.52 – 5.5 mg Co/L (*Daphnia magna*)
Algae toxicity	--	30.5 – 80.5 mg/L (*Navicula seminulum*, 96-h EC50)	0.52 mg Co/L (*Chlorella vulgaris*, 96-h EC50)

SIDS ENDPOINT	REPORTED VALUES		
	Cobalt naphthenate	**Naphthenic acids**	**Cobalt chloride**
Human Health Effects			
Acute Oral LD50	2,800 mg/kg and 2,828 mg/kg (two studies with rats)	5.88 g/kg (rat); 3.55 g/kg (mouse)	19.8 – 190 mg Co/kg (rat); 89.3 mg Co/kg (mouse)
Inhalation LC50	--	--	--
Dermal LD50	Between 1260 and 2000 mg/kg (rabbit)	> 3.14 g/kg (rabbit)	Increased proliferation of lymphatic cells at 9.6 – 14.7 mg Co/kg-day (various spp.)
Skin irritation	Slightly irritating (rabbit)	Moderately to severely irritating (rabbit)	Allergic dermatitis seen in humans
Eye irritation	Mild or slightly irritating (rabbit)	Moderate irritant (rabbit)	--
Repeated dose	--	60 mg/kg/day LOAEL for rats (physical, clinical and pathological changes)	4 mg Co/kg LOAEL for rats (organ weight changes); 0.6 mg Co/kg NOAEL for rats (blood parameter changes); LOAELs 0.5 – 30.2 mg Co/kg-day for rats in various studies
Genetic toxicity *(in vitro)*	Predominantly negative in Ames Assay. Negative in mouse lymphoma assay	Calcium and sodium naphthenate negative in Ames Assay; sodium naphthenate negative for chromosome aberrations but positive for sister chromatid exchanges; calcium naphthenate positive in mouse lymphoma assay	Co(2+) generally non-mutagenic in most bacterial assays; weak positive response with Chinese hamster V9 cells; DNA damage in human lymphocytes
Genetic toxicity *(in vivo)*	--	--	Clastogenic effects in mice

SIDS ENDPOINT	REPORTED VALUES		
	Cobalt naphthenate	**Naphthenic acids**	**Cobalt chloride**
Developmental	--	No developmental effects at 60/mg/kg/day in rats	NOAEL = 24.8 mg/kg/day in rats; 81.7 mg Co/kg in screening study (mice)
Reproductive	--	60 mg/kg/day oral dose produced effects on female fertility and total cholesterol in rats. No adverse effects on reproductive performance due to dermal exposure in male rabbits.	Effects in rats at 13.2 – 30.2 mg Co/kg/day; in mice at 23 – 58.9 mg Co/kg/day

[1] References are given in the robust summaries (Appendixes A – C)
[2] NR = not relevant

TEST PLAN AND RATIONALE FOR COBALT NAPHTHENATE

Cobalt Naphthenate	CASRN 61789-51-3

The Test Plan for cobalt naphthenate is presented in Table 4 with supporting data for the dissociation products. The rationale for the Test Plan is based upon existing data as summarized in the previous section and in Table 3.

Physicochemical Properties

Data is available for all five SIDS endpoints listed in Tables 3 and 4 for either the cobalt naphthenate salt or naphthenic acid, if not both. The vapor pressure endpoint is considered not applicable for the salt. The rationale for not conducting an octanol/water partition coefficient study with cobalt naphthenate is based on the impurity of the compound (i.e. a salt), and an ionizeable substance. Using a compound with these characteristics to measure the partition coefficient is inappropriate as it would yield erroneous data.

- No additional testing is recommended or proposed for any of the physico-chemical properties.

Environmental Fate Parameters

A GLP study was conducted to determine the dissociation constant for cobalt naphthenate. This is a key property, because the fate and effects of the compound are based upon the dissociation products. Of these dissociation products, environmental fate endpoints such as photodegradation or biodegradation are not relevant for cobalt chloride because this is a simple compound that releases the element cobalt which does not degrade further. For naphthenic acids, experimental data are available concerning both photodegradation and biodegradation. The values for the transport endpoint were predicted using EPIWIN for a mixture of naphthenic acids. Standard models used for estimating transport do not accurately predict salts or ionized substances and were not used for cobalt naphthenate.

- No additional testing is recommended or proposed for environmental fate properties of cobalt naphthenate. The potential biodegradation of cobalt naphthenate can be assessed based upon the available biodegradation studies for naphthenic acid, in conjunction with studies on other cobalt carboxylates being conducted by the Metals Carboxylates Coalition.

Ecotoxicity

No ecotoxicity studies are available for cobalt naphthenate. Data are available for both of the dissociation products for fish, invertebrates, and algae. However, the available data on naphthenic acids are from older, non-guideline studies with non-standard species.

- Acute toxicity tests with fish, daphnids and algae are proposed for cobalt naphthenate.

Human Health Effects

Acute toxicity studies

Acute toxicity data are available for four of the five endpoints (oral toxicity, dermal toxicity, skin irritation, and eye irritation) for cobalt naphthenate. Data are available for the same four endpoints for naphthenic acids, and the acute toxicity of cobalt chloride is well-characterized.

- No additional testing is recommended or proposed for acute toxicity endpoints.

Genotoxicity studies

Cobalt naphthenate was generally negative in the Ames assay and was negative in the mouse lymphoma assay. Calcium and sodium salts of naphthenic acid have shown mixed results in various *in vitro* genotoxicity studies. The genotoxicity of cobalt chloride has been well-characterized, including both *in vitro* and *in vivo* tests, with some negative and some positive responses.

- A genotoxicity test (the mouse micronucleus test) is proposed for cobalt naphthenate for the *in vivo* endpoint.

Higher tiered studies

There are no repeated dose studies available for cobalt naphthenate. An oral 90-day subchronic study was conducted with female rats on a mixture of naphthenic acids (sodium salts). Various repeated dose studies have been conducted with cobalt chloride.

- A combined repeated dose with repro/developmental screen (OECD 422) is proposed for cobalt naphthenate.

Table 4. Test Plan Matrix: Cobalt naphthenate

Data elements	Cobalt naphthenate			Naphthenic acids			Cobalt chloride			Testing recommended for cobalt naphthenate
	Information available	GLP study	Acceptable	Information available	GLP study	Acceptable	Information available	GLP study	Acceptable	
PHYSICOCHEMICAL PROPERTIES										
Melting Point	Y	N	Y	Y	N	Y	Y	N	Y	N
Boiling Point	Y	N	Y	Y	N	Y	Y	N	Y	N
Vapor pressure	NR			Y	N	Y	NA			N
Partition Coefficient	NR			Y	N	Y	NA			N
Water Solubility	Y	Y	Y	Y	N	Y	Y	N	Y	N
ENVIRONMENTAL FATE PARAMETERS										
Photodegradation	N			Y	N	Y	NA			N
Dissociation in water	Y	Y	Y	Y	N	Y				N
Transport	NR			Y	N	Y	NA			N
Biodegradation	N			Y	N	Y	NA			N
ECOTOXICITY										
Fish toxicity (96-h)	N			Y	N	Y	Y	N	Y	Y
Invertebrate toxicity (48-h)	N			Y	N	N	Y	N	Y	Y
Algae toxicity (72-h)	N			Y	N	N	Y	N	Y	Y
HUMAN HEALTH EFFECTS										
Acute										
Oral LD50	Y	N	Y	Y	N	Y	Y	N	Y	N
Inhalation LC50	N			N			N			N
Dermal LD50	Y	N	Y	Y	N	Y	N			N
Skin Irritation	Y	N	Y	Y	N	Y	Y	N	Y	N
Eye Irritation	Y	N	Y	Y	N	Y	N			N
Repeated dose	N			Y	N	Y	Y	N	Y	Y[c]
Genetic Toxicology – mutation assay	Y	N	Y	Y	Y	Y	Y	N	Y	N
Genetic Toxicology – chromosomal aberration	Y	N	Y	Y	Y	Y	Y	N	Y	N
Genetic Toxicology – *in vivo*	N			N			Y	N	Y	Y
Reproductive	N			Y	N	Y	Y	N	Y	Y[c]
Developmental	N			Y	N	N	Y	N	Y	Y[c]

[A] NR = not relevant; [b] NA = not available; [c] OECD 422 proposed

REFERENCES

Ashmead, H., D. Graff, and H. Ashmead, 1985. Intestinal absorption of metal ions and chelators. Charles C. Thermon, ed., Chicago, pp. 13-1996. As referenced in Firriolo et al., 1999.

ATSDR, 2001. Draft Toxicological Profile for Cobalt, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), September 2001.

Clemente, J.S. and P.M. Fedorak, 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60:585-600.

Firriolo, J.M., 1992. Disposition and toxicity after oral and intravenous administration of cobalt naphthenate and cobalt chloride in rats. Ph.D. Dissertation, University of Arizona.

Firriolo, J.M., F. Ayala-Fierro, I.G. Sipes, and D.E. Carter, 1999. Absorption and disposition of cobalt naphthenate in rats after a single oral dose. J. Toxicol. Environ. Health, Part A, 58:383-395.

Headley, J.V. and D.W. McMartin, 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, Vol. A39, No. 8, 00.1989-2010.

Herman et al. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. 40:467-477.

Lezotte, F.J. and W.B. Nixon, 2002. Determination of the dissociation constant of naphthenic acids, cobalt salts, Wildlife International, Ltd. Study No. 534C-109, conducted for the Metal Carboxylates Coalition.

Speijers, G.J.A., E.I. Krajnc, J.M. Berkvens, and M.J. van Logten, 1982. Acute oral toxicity of inorganic cobalt compounds in rats. Fd. Chem. Toxic. 20:311-314.

Stopford, W., J. Turner, D. Cappellini, and T. Brock (2003). "Bioaccessibility Testing of Cobalt Compounds." J. Environ Monit., 5:675-680.

U.S. Environmental Protection Agency, 2002. Draft Action Plan, Development of a Framework for Metals Assessment and Guidance for Characterizing and Ranking Metals. U.S. Environmental Protection Agency, EPA/630/P-02/003A, External Draft Review (June 2002).